**SUPPLEMENTARY MATERIAL**

**Biological activities of organic extracts of four *Aureobasidium pullulans* varieties isolated from extreme marine and terrestrial habitats**

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**Abstract:** We report on the screening for biological activities of organic extracts from seven strains that represent four varieties of the fungus *Aureobasidium pullulans*, i.e. *A. pulluans* var. *melanogenum*, *A. pullulans* var. *pullulans,* *A. pullulans* var. *subglaciale* and *A. pullulans* var. *namibiae*. We monitored hemolysis, cytotoxicity, antioxidant capacity, and growth inhibition against three bacterial species. The hemolytic activity of *A. pullulans* var. *pullulans* strain, EXF-150, was due to five different hemolytically active fractions. Extracts from all of the other varieties contained at least one hemolytically active fraction. Short-term exposure of cell lines to these hemolytically active organic extracts resulted in more than 95% cytotoxicity. Strong antioxidant capacity, corresponding to 163.88 µg ascorbic acid per gram total solid, was measured in the organic extract of the strain EXF-3382, obtained from *A. pullulans* var. *melanogenum*, isolated from the deep sea. Organic extracts from selected varieties of *A. pullulans* showed weak antibacterial activities.

Table S1. Retention factors following TLC separation of the chloroform and methanol extracted fractions from the *A. pullulans* varieties. The metabolites that showed hemolytic activity are marked in bold.

|  |  |  |  |
| --- | --- | --- | --- |
| **Variety** | **Growth medium** | **Solvent** | **TLC retention factor (Rf) of the separated fractions**  |
| *A. pullulans* var. *melanogenum* EXF-924 | MEA | CHCl3 | 0.01 | 0.03 | 0.05 | 0.18 | 0.39 | 0.51 | 0.56 | 0.67 | 0.80 |  |  |  |  |  |  |  |  |
| MeOH | **0.08** | 0.35 | 0.40 | 0.46 | 0.60 | 0.64 | 0.68 | 0.76 | 0.83 |  |  |  |  |  |  |  |  |
| MEA+10% NaCl | CHCl3 | 0.01 | 0.02 | 0.05 | 0.38 | 0.66 | 0.78 |  |  |  |  |  |  |  |  |  |  |  |
| MeOH | 0.02 | 0.07 | 0.63 | 0.78 | 0.85 |  |  |  |  |  |  |  |  |  |  |  |  |
| *A. pullulans* var. *melanogenum* EXF-3382 | MEA+10% NaCl | CHCl3 | 0.24 | 0.27 | 0.29 | 0.45 | 0.49 | 0.67 | 0.78 | 0.85 |  |  |  |  |  |  |  |  |  |
| MeOH | 0.14 | 0.25 | 0.29 | 0.31 | 0.43 | 0.49 | 0.53 | 0.62 | 0.64 | 0.81 | 0.87 |  |  |  |  |  |  |
| *A. pullulans* var. *namibiae* EXF-3398 | MEA | CHCl3 | 0.10 | 0.29 | 0.74 | 0.86 |  |  |  |  |  |  |  |  |  |  |  |  |  |
| MeOH | 0.07 | 0.11 | **0.32** | 0.50 | 0.55 | 0.71 | 0.83 |  |  |  |  |  |  |  |  |  |  |
| *A. pullulans* var. *pullulans* EXF-150 | MEA | CHCl3 | **0.06** | **0.10** | 0.14 | 0.46 | 0.49 | **0.56** | **0.66** | **0.71** | 0.79 | 0.87 | 0.92 |  |  |  |  |  |  |
| MeOH | **0.08** | **0.45** | **0.51** | **0.53** | 0.56 | 0.70 | **0.78** | 0.86 | 0.91 |  |  |  |  |  |  |  |  |
| *A. pullulans* var. *pullulans* EXF-1668 | MEA | CHCl3 | 0.01 | 0.03 | 0.03 | 0.06 | 0.30 | 0.47 |  |  |  |  |  |  |  |  |  |  |  |
| MeOH | 0.08 | 0.18 | 0.30 | 0.41 | 0.45 | 0.47 | 0.59 | 0.68 | 0.79 | 0.84 | **0.88** |  |  |  |  |  |  |
| MEA+10% NaCl | CHCl3 | 0.01 | 0.02 | 0.05 | 0.07 | 0.38 | 0.46 | 0.49 |  |  |  |  |  |  |  |  |  |  |
| MeOH | 0.07 | 0.23 | 0.38 | 0.44 | 0.61 | 0.77 | 0.83 |  |  |  |  |  |  |  |  |  |  |
| *A. pullulans* var. *subglaciale* EXF-2481 | MEA | CHCl3 | **0.07** | 0.33 | 0.44 | 0.82 |  |  |  |  |  |  |  |  |  |  |  |  |  |
| MeOH | 0.29 | 0.32 | 0.46 | 0.59 | 0.73 | **0.83** | 0.90 |  |  |  |  |  |  |  |  |  |  |
| MEA+10% NaCl | CHCl3 | 0.28 | 0.35 | 0.41 | 0.46 | 0.50 | 0.54 | 0.61 | 0.66 | 0.78 | 0.83 | 0.87 |  |  |  |  |  |  |
| MeOH | 0.12 | 0.17 | 0.22 | 0.27 | 0.34 | 0.39 | 0.42 | 0.45 | 0.47 | 0.50 | 0.51 | 0.55 | 0.59 | 0.63 | 0.75 | 0.82 | 0.87 |
| *A. pullulans* var. *subglaciale* EXF-2479 | MEA+10% NaCl | CHCl3 | 0.14 | 0.23 | 0.29 | 0.45 | 0.48 | 0.53 | 0.58 | 0.61 | 0.66 | 0.74 | 0.83 | 0.89 |  |  |  |  |  |
| MeOH | 0.08 | 0.11 | 0.13 | 0.20 | 0.31 | 0.38 | 0.39 | 0.41 | 0.45 | 0.49 | 0.55 | 0.58 | 0.62 | 0.69 | 0.83 |  |  |

MEA: malt extract agar; MEA+10% NaCl: malt extract agar with the addition of 10% NaCl.



Figure S1.Bioautographic method for antioxidant capacity determination. The DMSO-dissolved organic extracts of the selected *A.* *pullulans* varieties containing metabolites with the potential to scavenging DPPH, appear as yellow spots against a purple background, and are compared to the positive control (ascorbic acid; c = 1.67 mg/mL), which has well-defined antioxidant activity. The solvent (DMSO) was used as the negative control.

Table S2. Details of the *A.* *pullulans* varieties used in the present study.

|  |  |  |  |
| --- | --- | --- | --- |
| **Variety** | **Strain accession No.** | **Origin** | **Liquid growth medium** |
|  |  |  | **MEA** | **MEA + 10% NaCl** |
| *Aureobasidium pullulans* var. *melanogenum* | EXF-924 | Ponds on sea ice, Svalbard, Kongsfjorden, Norway | + | + |
|  | EXF-3382 | Deep sea, 4500 m n depth, Japan | NG | + |
| *Aureobasidium pullulans* var. *namibiae* | EXF-3398 | Dolomitic marble, Namibia Desert, Africa | + | NG |
| *Aureobasidium pullulans* var. *pullulans* | EXF-150 | Hypersaline saltern water, Sečovlje Salterns, Slovenia | + | NG |
|  | EXF-1668 | Glacial ice from sea water, Svalbard, Conwaybreen, Kongsvegen, Norway | + | + |
| *Aureobasidium pullulans* var. *subglaciale* | EXF-2481 | Subglacial ice from sea water, Svalbard, Kongsvegen, Norway | + | + |
|  | EXF-2479 | Glacial ice from sea water, Svalbard, Kongsvegen, Norway | NG | + |

NG: no growth was observed; MEA: malt extract agar; MEA+10% NaCl: malt extract agar with the addition of 10% NaCl.

Table S3. Total solids of the organic extracts obtained from the *Aureobasidium* varieties, as dissolved in DMSO.

|  |  |  |  |
| --- | --- | --- | --- |
| **Variety** | **Growth medium** | **Solvent** | **Total solids** (mg/mL) |
| *A. pullulans* var. *melanogenum* EXF-924 | MEA | CHCl3 | 2.32 |
| MeOH | 3.28 |
| MEA+10%NaCl | CHCl3 | 1.84 |
| MeOH | 5.24 |
| *A. pullulans* var. *melanogenum* EXF-3382 | MEA+10%NaCl | CHCl3 | 0.2 |
| MeOH | 2.88 |
| *A. pullulans* var. *namibiae* EXF-3398 | MEA | CHCl3 | 0.24 |
| MeOH | 1.4 |
| *A. pullulans* var. *pullulans* EXF-150 | MEA | CHCl3 | 0.81 |
| MeOH | 0.92 |
| *A. pullulans* var. *pullulans* EXF-1668 | MEA | CHCl3 | 0.16 |
| MeOH | 0.08 |
| MEA+10%NaCl | CHCl3 | 0.08 |
| MeOH | 0.2 |
| *A. pullulans* var. *subglaciale* EXF-248 | MEA | CHCl3 | 0.24 |
| MeOH | 1.72 |
| MEA+10%NaCl | CHCl3 | 0.4 |
| MeOH | 4.44 |
| *A. pullulans* var. *subglaciale* EXF-2479 | MEA+10%NaCl | CHCl3 | 0.32 |
| MeOH | 5.72 |

MEA: malt extract agar; MEA+10% NaCl: malt extract agar with addition of 10% NaCl.



Figure S2. Schematic presentation of the experimental set-up used in the present study.